

**Citation:**

Crujeiras AB, Parra D, Abete I, Martínez JA. A hypocaloric diet enriched in legumes specifically mitigates lipid peroxidation in obese subjects. *Free Radic Res*. 2007 Apr; 41 (4): 498-506.

**PubMed ID:** [17454132](#)

**Study Design:**

Randomized Controlled Trial

**Class:**

A - [Click here](#) for explanation of classification scheme.

**Research Design and Implementation Rating:**

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

To determine if a hypocaloric diet high in non-soybean legumes would decrease oxidative stress in obese subjects in addition to the known effects associated with weight loss, especially in relation to lipid peroxidation.

**Inclusion Criteria:**

- Obese men and women
- Good health history as determined by physical examination and routine laboratory tests.

**Exclusion Criteria:**

- Weight fluctuation at least 3kg in the past three months
- Taking prescription medication in the last three months
- Taking supplemental vitamins or minerals during the last three months.

**Description of Study Protocol:****Recruitment**

Recruitment was through local advertisements

**Design**

- Subjects were randomized into two groups
- Before and after nutritional intervention body weight was measured and fasting blood and eight-hour urine samples were taken
- Total cholesterol (TC) and oxidative stress markers were measured at baseline and after diet intervention.

## **Dietary Intake/Dietary Assessment Methodology**

- Dietary compliance was monitored using three-day weighted diet diaries
- Total antioxidant capacity (TAC) was calculated using the total antioxidant concentration of each food.

## **Blinding Used**

Though not explicitly stated, it is assumed that the laboratory assessments were not correlated by group and therefore essentially blinded.

## **Intervention**

- Each group received a hypocaloric diet for eight weeks which was designed to produce a caloric restriction of -30% as determined by individual measurement of resting energy expenditure by indirect calorimetry
- Control group did not receive legumes in the diet
- Study group received non-soybean legumes four days per week
- The macronutrient distributions of both diets were similar
  - 50% energy from carbohydrates (CHO)
  - 20% energy from protein
  - 30% energy from lipids.

## **Statistical Analysis**

Sample size was determined based on published values of the standard deviation (SD) for serum malondialdehyde (MDA)

- Student T-test was used to detect differences before and after weight loss and the differences between diet response
- Pearson coefficient was used to determine associations between weight loss and changes in total cholesterol and oxidative stress markers
- Spearman test was used to identify associations between biochemical variables and dietary TAC and fiber
- Multivariate linear regression models were used to explain differences in oxidative stress biomarkers in relation to diet or TAC adjusted for energy intake, weight loss and total cholesterol changes
- Since there was no statistical differences between dietary groups ( $P=0.10$ ) regression models were not used to adjust for possible confounders of gender, body mass index (BMI) or age.

## **Data Collection Summary:**

### **Timing of Measurements**

Measurements were taken at baseline and after eight weeks of diet modification.

### **Dependent Variables**

- Variable 1: Weight
- Variable 2: TC, HDL-C, triglycerides (TG), glucose, uric acid, and total bilirubin were measured by calorimetric assays, plasma LDL-C data were calculated using the Friedewald equation

- Variable 3: MDA and total plasma antioxidant power were measured using commercial calorimetric assay kits
- Variable 4: Insulin concentrations were determined using a radioimmunoassay method
- Variable 5: Plasma level of circulating oxidized LDL and urinary excretion of 8-isoprostan  $F_2\alpha$  were assessed by enzyme-linked immunosorbent assay kits and were expressed per milligram of creatinine.

### Independent Variables

Hypocaloric diet with non-soybean legumes consumed four days per week.

### Control Variables

- Macronutrient distribution of diet
- Cholesterol content of the diet
- TAC of diets
- Fiber content of diets.

### Description of Actual Data Sample:

- *Initial N*: 17 men and 13 women
- *Attrition*: None
- *Mean age*:  $36 \pm 8$  years
- *Ethnicity*: Not identified
- *Other relevant demographics*: None
- *Anthropometrics*: Mean BMI  $32 \pm 5.3 \text{ kg/m}^2$
- *Location*: University Clinic of Navarra, Spain.

### Summary of Results:

#### Changes in Variables in Response to Calorie Restricted Intervention

	Intervention Related Changes (N=30)		
	Baseline	End-point	P-value
<b>Weight (kg)</b>	93.4 $\pm$ 14.5	87.3 $\pm$ 13.7	<0.001
<b>BMI (kg/m<sup>2</sup>)</b>	32.4 $\pm$ 4.5	30.4 $\pm$ 4.3	<0.001
<b>Total cholesterol (mg/dL)</b>	199 $\pm$ 39	178 $\pm$ 29	<0.001
<b>Glucose (mg/dL)</b>	95 $\pm$ 8	92 $\pm$ 6	0.847
<b>Insulin (<math>\mu</math>U/ml)</b>	9.42 $\pm$ 7.90	7.36 $\pm$ 4.1	0.290
<b>Oxidized LDL (I/L)</b>	115 $\pm$ 67	110 $\pm$ 58	0.569

#### Changes in Weight and Oxidative Markers by Diet Intervention

	Control Diet (N=15)	Legume-based Diet (N=15)
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	Baseline	End-point	P-value	Baseline	End-point	P-value
<b>Body weight (kg)</b>	92.5±13.2	87.7±13.0	<0.001	94.4±16.	87.0±14.7	<0.001
<b>Total cholesterol (mg/dL)</b>	181±35	173±32	0.140	215±37	182±27	<0.001
<b>Oxidized LDL (U/L)</b>	109±56	121±67	0.260	121±78	99±46	0.091

### Other Findings

- Mean caloric intake was 2,479±1,832kcal per day at baseline, and 1,462±354kcal per day at the end-point (P=0.001)
- There was a significant decrease in body weight in both groups following the energy-restricted diets, with higher weight loss in the LD group as compared to the CD (-7.7±3% vs. -5.3±2.7%; P=0.023). The decrease in body weight correlated with the dietary fiber content (r=0.46; P=0.014)
- The diets did not have statistical differences in cholesterol content (P=0.641); fiber content in the LD diet was statistically higher than in the CD diet (25±6 vs. 18±5g per day; P=0.005). Total plasma cholesterol concentrations decreased in both diets, being significantly different between both diet groups (-14.4±10.6 vs. -3.9±10.7%; P<0.001). The decrease in TC was directly correlated with body weight loss (r=0.50; P=0.006) and with increased fiber intake (r=0.44; P=0.022)
- Changes in Ox-LDL, MDA and 8-iso-PGF<sub>2α</sub> while significant in the LD group comparison of both groups did not show statistical significantly differences in the two groups
- Multiple regression analysis were preformed to explore dietary effects on oxidative stress markers
  - 26% of variability in total cholesterol changes were explained by both weight loss and type of diet
  - ox-LDL concentration showed a 25% decrease dependant on total cholesterol changes and in relation with dietary TAC and weight loss
  - The MDA decrease was explained by changes in TC concentrations when adjusted for MDA baseline
  - Linear regression analyses to explain differences in 8-iso-PGF<sub>2α</sub> were related to the estimated dietary TAC for decreasing the levels of this marker.

### Author Conclusion:

The authors conclude that inclusion of non-soybean legumes four days per week with a moderate calorie restriction is able to ameliorate oxidative stress associated to lipids.

### Reviewer Comments:

- *The quantity of legumes consumed four times per week is not specified*
- *It is unclear if the control group ate any legumes or were told to avoid them*
- *Repeated measures ANOVA may have been more appropriate statistical analysis for some of the outcomes (e.g., body weight and cholesterol).*

## Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes
Validity Questions		
1.	<b>Was the research question clearly stated?</b>	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	<b>Was the selection of study subjects/patients free from bias?</b>	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	<b>Were study groups comparable?</b>	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	No
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes

3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
<b>4.</b>	<b>Was method of handling withdrawals described?</b>	N/A
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
<b>5.</b>	<b>Was blinding used to prevent introduction of bias?</b>	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	???
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
<b>6.</b>	<b>Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?</b>	No
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	No
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A

6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
<b>7.</b>	<b>Were outcomes clearly defined and the measurements valid and reliable?</b>	<b>Yes</b>
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	???
7.7.	Were the measurements conducted consistently across groups?	Yes
<b>8.</b>	<b>Was the statistical analysis appropriate for the study design and type of outcome indicators?</b>	<b>Yes</b>
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes

8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
<b>9.</b>	<b>Are conclusions supported by results with biases and limitations taken into consideration?</b>	<b>Yes</b>
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
<b>10.</b>	<b>Is bias due to study's funding or sponsorship unlikely?</b>	<b>Yes</b>
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes